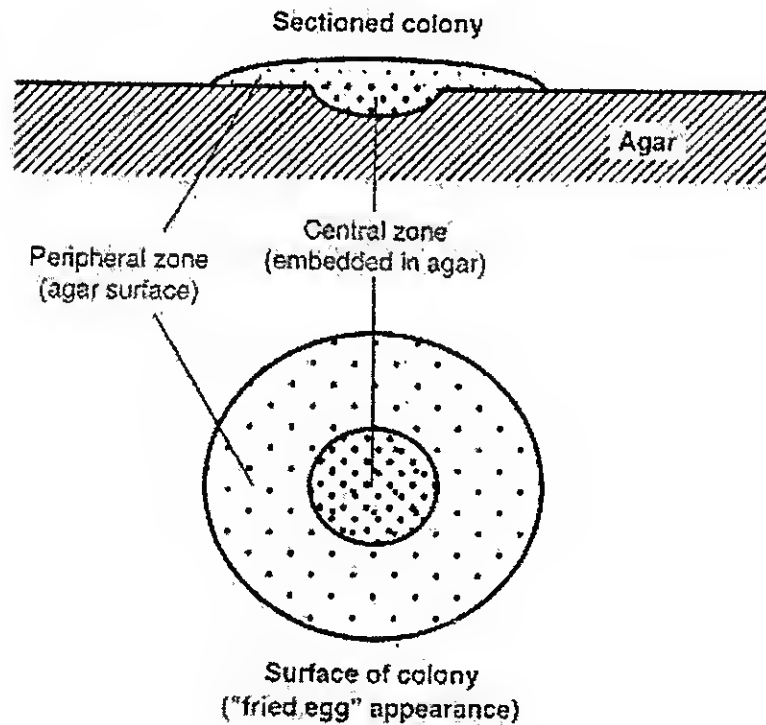


Gram – ve Bacteria

Class: Mollicutes (Mycoplasmas)



أ.د/ جمال يونس

Phylum(Division): Firmicutes

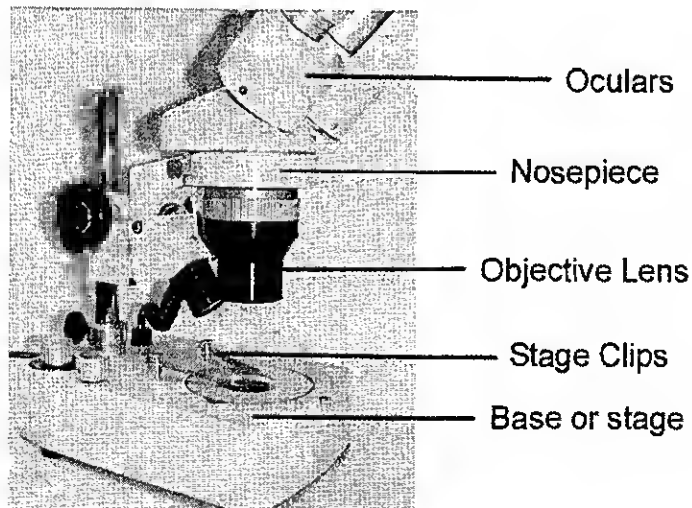
CLASS : MOLLICUTES

Family: Mycoplasmataceae

Recent Taxonomic classification of Mycoplasmas:

under phylum (division) Firmicutes due to low GC – content (18 – 40 mol %) small genome.

Dissecting Microscope



Class: Mollicutes

- ♦ Lack cell wall and do not synthesize peptidoglycan.
- ♦ Mollicutes are bacteria which have small genomes, Lack a cell wall and have a low GC-Content (18 - 40 mol %)

Classification:

order: Mycoplasmatales

Family: Mycoplasmataceae
(Choleplasmataceae)

Genus I: Mycoplasma

Species:

a-avian mycoplasmas:

- ♦ M. gallisepticum
- ♦ M. meleagridis
- ♦ M. synoviae
- ♦ M. iowae

b-animal mycoplasmas:

- ♦ M. mycoides
- ♦ M. bovis
- ♦ M. bovirhinum
- ♦ M. bovoculi

order: Acholeplasmatales

Family: Acholeplasmataceae
(does not need cholesterol for its growth)

Genus: Acholeplasma

Genus II: Ureaplasma

(require urea for its growth)

pathogenicity:

Species	disease
(a) avian mycoplasmas:	
♦ M. gallisepticum	avian mycoplasmosis ♦ CRD in chickens (with other bacteria mainly E. coli) ♦ Infectious sinusitis in turkeys.
♦ M. meleagridis	air-sacculitis and bursitis in turkeys
♦ M. synoviae	Synovitis in chicken and turkeys
♦ M. iowae	air-sacculitis, stunting and Leg deformities.
N.B: These 4 species are <u>egg transmitted</u> .	
(b) animal mycoplasmas:	
1- M. mycoides	
♦ subsp. mycoides	CBPP (Contagious bovine pleuropneumonia) in cattle.
♦ subsp. Capri	CCPP (Contagious Caprine pleuropneumonia) in goats.
2- M. bovis	Mastitis and arthritis in cattle
3- M. bovis genitalium	Vaginitis, arthritis, mastitis and Seminal Vesiculitis in cattle
4- M. bovoculi	Keratoconjunctivitis in cattle
5- M. agalactiae	Contagious agalactia in sheep and goats
6- M. ovipneumoniae	pneumonia in sheep.

general characters of Mycoplasmas :

- ① Gram -ve bacteria
- ② stain poorly with Gram's stain but they produce better results with Giemsa and Romanowsky stains.
- ③ IntraCellular bacteria
- ④ They are devoid of cell wall peptidoglycan
→ So, they have not constant shape (pleomorphic).
- ⑤ The Cytoplasm is surrounded by plasma membrane which is a triple-layered and contain sterol (unit-membrane).
- ⑥ They are the smallest bacteria (0.1-0.3 μm in diameter).
- ⑦ Non-motile
- ⑧ growth requirements:
 - 1 - O_2 requirement:
 - ◆ aerobic or facultative anaerobic
 - ◆ Some of them require 5% CO_2 to grow (capnophilic)
 - ◆ others are strict anaerobic (called anaeroplasm)
 - 2 - opt. temp for growth → 35 - 37°C
 - 3 - opt. pH → 7.2 - 7.8
 - 4 - They need :
 - ◆ high conc. of Serum (10-15%), cholesterol or NAD Factor to grow.

- Inhibitors e.g.
 - penicillin (KILL G⁺ve bacteria)
 - thalium acetate (KILL G⁻ve)
 - amphotericin B (KILL fungi)

This is because mycoplasmas Lack cell wall
 → so, they are resistant to penicillin that
 used for the inhibition of contaminants.

5. Media used:

- Mycoplasma agar and broth
- pplo media

→ produce Fried egg micro-colonies

- They are the smallest colonies (0.1-0.6 mm in diameter) → visible under dissecting or stereomicroscope or low power of ordinary microscope or hand lens.

- The micro-colonies are ch' by → an opaque granular center with a deep growth in the medium and a flat transparent zone of growth at periphery.

flat transparent zone of growth at periphery

opaque granular center

deep growth in the medium

Laboratory diagnosis :

① Isolation :

◆ O₂ requirement :

- aerobic or facultative anaerobic
- Some require 5% CO₂ to grow (Capnophilic)

◆ opt. temp. → 35 - 37 °C

◆ opt. pH → 7.2 - 7.8

◆ Incubation time :

The growth is slow → so, incubation of cultures should be continued from 2-4 days to 14 days in a humid atmosphere before being regarded as -ve.

◆ Specific media of mycoplasmas :

- Mycoplasma agar and broth
- heart infusion broth and agar
- ppLo media

→ Contain inhibitors :

- penicillin (kill Gram +ve bacteria)
- Thallium acetate (kill Gram -ve bacteria)
- amphotericin-B (to kill fungi)

◆ obtaining a pure culture is done by culturing either on

- agar - broth - agar
- agar - agar

② Identification:

a. Culture characters:

- on Liquid media → slight turbidity
- on Solid media → fried egg micro-colonies
- differentiation between mycoplasma micro-colonies and bacterial L-forms:

1. subculture on media without antibacterial substances (up to 5 subcultures) → Mycoplasma micro-colonies remain very small.

2. stain the micro-colonies with Dienes's stain →

→ Mycoplasma micro-colonies retain the stain indefinitely.

→ bacterial L-forms colonies → decolourize in about 15 min.

b. Morphology:

of little value due to:

1. weak staining by various methods.
2. pleomorphism (Mycoplasma is an originally pleomorphic organism because they lack cell wall and do not synthesize peptidoglycan)

c. Identification of the genus:

by digitonin test (sensitivity to digitonin)

Sensitive

(Inhibition of growth by digitonin)

↓
Modified urease test

↙
urease -ve

Mycoplasma
(Colonies →
0.1 - 0.6 mm in
diameter)

Resistant

(growth)

↓
Acholeplasma
(Colonies →
upto 3 mm in
diameter)

↘
urease +ve

ureaplasma
(Colonies →
0.01 - 0.05 mm in
diameter "Tiny or
T-mycoplasmas")

d. Identification of species:

① Biochemical tests: such as

- ♦ glucose fermentation
- ♦ arginine hydrolysis
- ♦ phosphatase activity
- ♦ Reduction of tetrazolium

② Serological tests (Sero-diagnosis):

a-For mycoplasmal antigens (using known antisera)

1- FAT (direct or Indirect)

used commonly with avian mycoplasmas

2- agar gel diffusion

3- ELISA

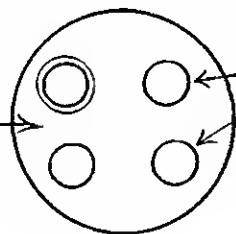
4- CFT

5- growth inhibition test:

growth inhibition test:

depend on the inhibition of growth of mycoplasma on solid media by the potent specific antisera.

solid media
inoculated
with the organism



paper discs
impregnated with
specific antisera
against different
serotypes.

+ve result:

Zone of growth inhibition around the disc

b-For mycoplasmal antibodies

(using known antigen)

1- plate (rapid) agglutination test

(Serum plate agglutination test, SpA)

→ using coloured stained killed Ag

→ For avian mycoplasmas

2 - Haemagglutination inhibition test (HI):

- For avian mycoplasmas
- more sensitive than SpA

3 - Indirect haemagglutination

4 - ELISA

5 - CFT

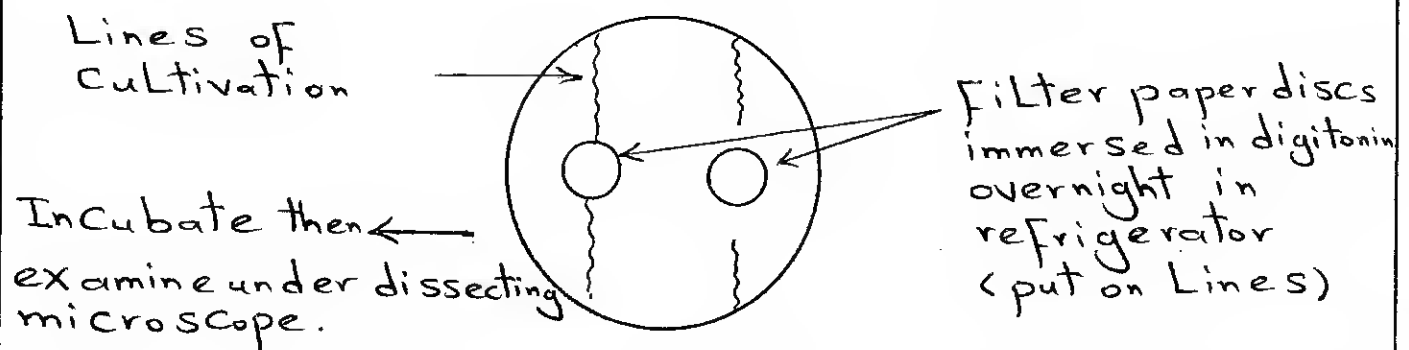
6 - agar gel diffusion test → for avian mycoplasma.

③ Molecular biological tests:

- ◆ PCR
- ◆ DNA probes

digitonin sensitivity test:

→ used to differentiate between Mycoplasma and acholoplasma (non-pathogenic)



◆ If no growth → Mycoplasma (sensitive to digitonin)

Susceptibility to antibiotics:

- Mycoplasmas are resistant to antibiotics which act on cell wall such as penicillins.
- They are sensitive to antibiotics which inhibit protein synthesis such as tetracyclins and erythromycin.

specific pathogen free (spf) programs:

to obtain poultry flocks free from the major avian mycoplasmas → by

- ① detection of infections and control of infected birds.
- ② periodic serological monitoring of the flocks to demonstrate continued freedom from infection.

Genus: Ureaplasma

U. urealyticum:

Cause non-gonococcal urethritis in man.

- Colonies → very very small (0.01-0.05 mm in diameter) → so, they are called T-mycoplasmas (Tiny mycoplasmas)
- require urea for its growth in addition to other requirements of Mycoplasma.

- pH \longrightarrow 6.0
- Thallium acetate \longrightarrow not used in the medium because it is harmful to ureaplasma species.

Modified urease test:

aim:

biochemical test used for differentiation between Mycoplasma and ureaplasma.

Technique:

Mixture of equal parts of 10% urea soln. + 0.8% manganese chloride \longrightarrow apply directly onto 40 hrs old microColonies



+ve result

Immediate colour reaction goes from Light to dark brown and finally to black (due to deposition of manganese on the surface of Colonies).

+ve result \longrightarrow ureaplasma
-ve result \longrightarrow Mycoplasma